

**Table S1. Strains and plasmids used in this study**

Strains or plasmids	Genotypes and/or descriptions <sup>a</sup>	Reference or source
<b>Strains</b>		
<i>P. aeruginosa</i>		
PAO1	Wild-type PAO1	B.H. Holloway
ΔsagS	PAO1 ΔsagS (PA2824)	(1)
ΔsagS::CTX	ΔsagS harboring the empty pMini CTX vector, Tet <sup>R</sup>	(2)
ΔsagS::CTX-sagS	ΔsagS harboring chromosomal insertion of sagS under the control of the sagS promoter at attB site, cured pMini CTX vector	(2)
ΔnicD	PAO1; PA4929::ISlacZ, Tet <sup>R</sup>	(3)
ΔPA3177	PAO1 ΔPA3177	(4)
<i>E. coli</i>		
DH5α	F <sup>-</sup> φ80lacZΔM15 Δ(lacZYA-argF)U169 recA1 endA1 hsdR17(r <sub>k</sub> <sup>-</sup> , m <sub>k</sub> <sup>+</sup> ) phoA supE44 thi-1 gyrA96 relA1 tonA	Life Technologies
<b>Plasmids</b>		
pJN105	Arabinose-inducible gene expression vector; pBRR-1 MCS; araC-P <sub>BAD</sub> , Gm <sup>R</sup>	(5)
pMJT-1	araC-P <sub>BAD</sub> cassette of pJN105 cloned into pUCP18, Amp <sup>R</sup> (Carb <sup>R</sup> )	(6)
pJN-sagS	C-terminal HA-tagged sagS cloned into pJN105 at NheI/Sacl, Gm <sup>R</sup>	(1)
pMJT-sagS	C-terminal HA-tagged sagS cloned into pMJT1 at NheI/Sacl, Amp <sup>R</sup> (Carb <sup>R</sup> )	(7)
pMJT-nicD-V5/6xHis	C-terminal V5/6xHis-tagged nicD cloned into pMJT1 at NheI/XbaI, Amp <sup>R</sup> (Carb <sup>R</sup> )	(8)
pMJT-nicDΔNoTMR-V5/6xHis	C-terminal V5/6xHis-tagged nicD lacking the DISMED2 sensory domain, cloned into pMJT1 at NheI/SmaI, Amp <sup>R</sup> (Carb <sup>R</sup> )	(8)
pCdrA::gfp(ASV)	pUCP22Not-P <sub>cdrA</sub> -RBS-CDS-RNase III-gfp(ASV)-T <sub>0</sub> -T <sub>1</sub> , Amp <sup>R</sup> , Gm <sup>R</sup>	(9)
pMF440	Broad host range plasmid for constitutive expression of mCherry, Amp <sup>R</sup> (Carb <sup>R</sup> )	Michael Franklin (Addgene plasmid #62550)

<sup>a</sup> Tet<sup>R</sup>, tetracyclin-resistant; Gm<sup>R</sup>, gentamicin-resistant; Amp<sup>R</sup>, ampicillin-resistant; Carb<sup>R</sup>, carbenicillin-resistant.

### Supplementary References

1. Petrova OE, Sauer K. 2011. SagS contributes to the motile-sessile switch and acts in concert with BfiSR to enable *Pseudomonas aeruginosa* biofilm formation. J Bacteriol 193:6614-6628.

2. Dingemans J, Al-Feghali RE, Lau GW, Sauer K. 2019. Controlling chronic *Pseudomonas aeruginosa* infections by strategically interfering with the sensory function of SagS. *Mol Microbiol* 111:1211-1228.
3. Jacobs MA, Alwood A, Thaipisuttikul I, Spencer D, Haugen E, Ernst S, Will O, Kaul R, Raymond C, Levy R, Chun-Rong L, Guenthner D, Bovee D, Olson MV, Manoil C. 2003. Comprehensive transposon mutant library of *Pseudomonas aeruginosa*. *Proc Natl Acad Sci U S A* 100:14339-14344.
4. Poudyal B, Sauer K. 2018. The PA3177 Gene encodes an active diguanylate cyclase that contributes to biofilm antimicrobial tolerance but not biofilm formation by *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 62:e01049-18.
5. Newman JR, Fuqua C. 1999. Broad-host-range expression vectors that carry the L-arabinose-inducible *Escherichia coli araBAD* promoter and the araC regulator. *Gene* 227:197-203.
6. Kaneko Y, Thoendel M, Olakanmi O, Britigan BE, Singh PK. 2007. The transition metal gallium disrupts *Pseudomonas aeruginosa* iron metabolism and has antimicrobial and antibiofilm activity. *J Clin Invest* 117:877-888.
7. Petrova OE, Gupta K, Liao J, Goodwine JS, Sauer K. 2017. Divide and conquer: the *Pseudomonas aeruginosa* two-component hybrid SagS enables biofilm formation and recalcitrance of biofilm cells to antimicrobial agents via distinct regulatory circuits. *Environ Microbiol* 19:2005-2024.
8. Basu Roy A, Sauer K. 2014. Diguanylate cyclase NicD-based signalling mechanism of nutrient-induced dispersion by *Pseudomonas aeruginosa*. *Mol Microbiol* 94:771-793.
9. Rybtke MT, Borlee BR, Murakami K, Irie Y, Hentzer M, Nielsen TE, Givskov M, Parsek MR, Tolker-Nielsen T. 2012. Fluorescence-based reporter for gauging cyclic di-GMP levels in *Pseudomonas aeruginosa*. *Appl Environ Microbiol* 78:5060-5069.